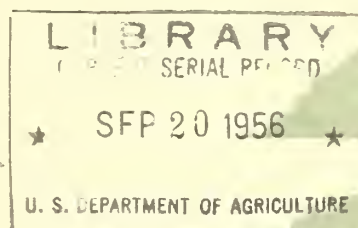


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Experimental Air-layering of Shortleaf and Loblolly Pine

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Closeup of air-layered stem of $2\frac{1}{2}$ -year-old shortleaf pine seedling, showing abundant root development 36 days after beginning of treatment.

EXPERIMENTAL AIR-LAYERING OF SHORTLEAF
AND LOBLOLLY PINE

by

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INTRODUCTION

Methods currently available for the vegetative propagation of the genus Pinus in forest genetics programs in the South and Southeast include grafting, rooting, and air-layering. Of these, only grafting has developed into a practical tool. It already is being used to establish seed orchards in Georgia and Florida with scion material from selected trees. But rooting and air-layering are in many ways more desirable forms of vegetative propagation, since, unlike grafting, they reproduce the genotype of the parent tree in both the top and root system of the new plant. Unfortunately, rooting for most species of pine is as yet difficult, and is therefore of limited use in experimental work. Air-layering of pine, as first used by Mergen (4) on slash pine, offers promise as a practical tool, at least on younger trees.

Methods of vegetative propagation whereby the root system of the parent tree can be multiplied are especially useful in studying the genotype of selected individuals. This is especially true in pathological studies where an attempt is made to incorporate resistance to a root induced disease. Where only the above-ground parts are attacked, such as in the white pine blister rust disease, it may be practical to graft scions from selected trees and test these "progeny" for resistance to infection (1).

Tree diseases such as littleleaf of pine (2), originating in the root system, are not amenable to such treatment. The causal factors of littleleaf include the soil fungus, Phytophthora cinnamomi, and associated adverse soil conditions. The root system itself must be tested against this complex in seeking an inherited resistance to littleleaf. Currently, selected trees are being subjected to examination by testing 1-year-old open- and control-pollinated seedlings (6). Information on the genotype of these parent trees could be obtained more accurately and rapidly by use of rooted material.

The basic simplicity of rooting cuttings makes this method appealing for genetics use. The method has found wide application in horticulture but only limited use in forest genetics research because of rather meager and erratic success with trees, especially pines. More complete understanding of the physiological and environmental conditions is needed if this method is to be pursued successfully. Furthermore, the factors involved in the effect of age

upon rootability are not well understood. Age of the parent tree determines how easily material from it will strike roots; shoots from trees beyond 10 years of age are rooted only with difficulty.

Attention has turned recently in the Southeast to the possible use of air-layering to supplement and even replace the rooting of cuttings from pine. The method has been used successfully by Mergen (4) with 5- to 17-year-old slash pines; he obtained 85 percent success using Hormodin No. 3^{1/} and 50 percent without this auxin. He did not compare rooting of the branches for the different ages used. Cech (3) had good results air-layering branches on 3- to 5-year-old loblolly pines. Beyond this age, root formation was difficult, although a few rooted air-layers were obtained from trees up to 60 years old.

The present study reports preliminary trials in air-layering shortleaf and loblolly pine, not for the practical purpose of vegetative propagation but rather to study factors involved in the rooting of cuttings. Elaborate experimental layouts involving several thousand cuttings and different treatments commonly fail to yield even one successfully rooted cutting. The greater and more uniform success of air-layering provides an opportunity for studying and evaluating responses to physiological and environmental treatments. The similarity between these two forms of vegetative propagation allows the ready transfer of conclusions with, of course, some reservation.

METHODS AND RESULTS

General

The techniques of air-layering as described by Mergen (4) and variations thereof were applied mainly to 2 $\frac{1}{2}$ -year-old potted shortleaf and loblolly seedlings. A few were made on branches of two shortleaf pine grafts made 8 years previously, using scion material from 35-year-old trees. In brief, the basic procedure was as follows: The needles were removed from the branch or main stem to be treated, and a $\frac{1}{2}$ -inch wide band of bark removed by first scoring the bark with a knife. Care was exercised to avoid cutting into the sapwood. (Air-layers often failed in first attempts because knife cuts were too deep and penetrated the sapwood.) The bark was then peeled off with the fingers. The exposed sapwood was not scraped and in no case was there a bridging of the gap by callus tissue. Auxin when applied was Hormodin No. 3. This was dusted with a small brush onto the upper cut bark surface. Next, the treated area was enveloped in a handful of moist sphagnum moss, which was then tightly encased in a sheath of polyethylene plastic. Finally, the whole was loosely wrapped in aluminum foil to reduce heat buildup within.

^{1/} 0.8 percent indolebutyric acid in talc.

Air-Layering in Trough

A modified method of applying sphagnum moss to the air-layered stem was tried. A wooden trough 6 in. x 6 in. x 10 ft. was made with loose bottom joints to allow drainage of excess water. At 6-inch intervals, opposite-aligned slots $\frac{1}{2}$ in. x 3 in. were cut downward in the sides. Moist sphagnum moss was packed in the bottom up to the lower level of the slots and the girdled seedling stems inserted horizontally. This permitted the foliage to be fully exposed to sunlight; the intact root system was mulched in sawdust. More sphagnum moss was added, completely filling the box. Finally, a cover board sheathed with aluminum foil was installed over the top (fig. 1). This was removed periodically for examination and for watering when necessary. The method gave excellent results.

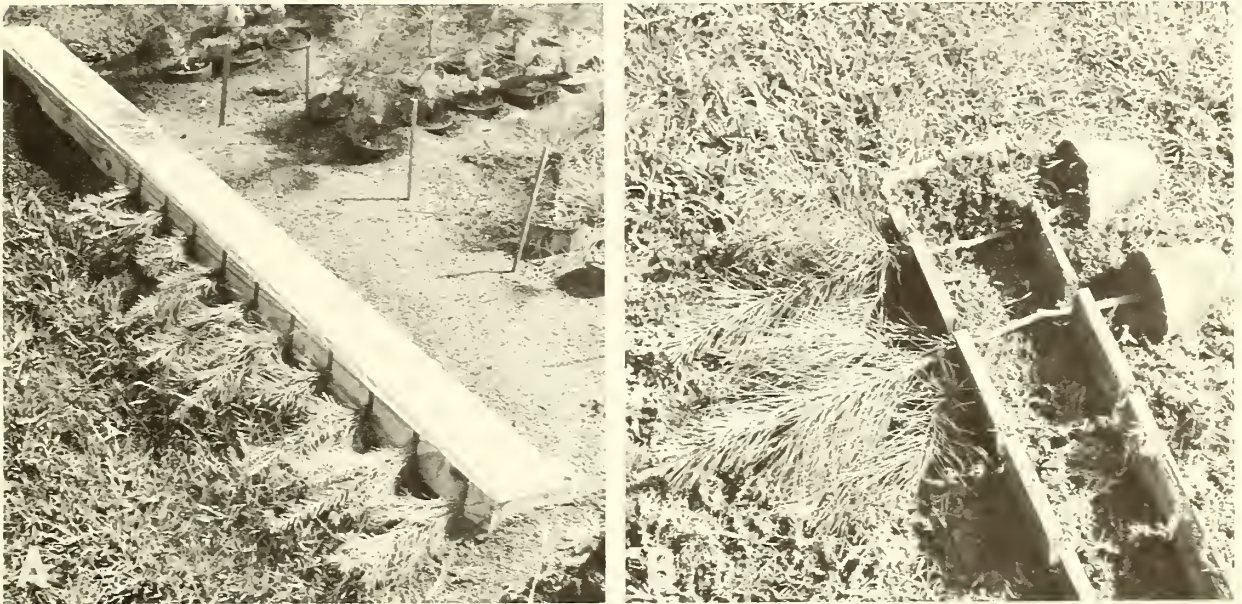


Figure 1.--Trough method of air-layering seedlings. A, Completed trough with seedlings in place and roots mulched in sawdust. B, Closeup of open trough with seedling stems inserted in slots.

Synthetic Sponge as Rooting Medium

A substitute for sphagnum moss as rooting medium was tried in air-layering the stems of upright seedlings. DuPont synthetic sponge was used with good results (fig. 2) by cutting two blocks 2 in. x $1\frac{1}{2}$ in. x $1\frac{1}{4}$ in. from a larger washing sponge and gouging out small cavities in each. After the sponge blocks had been wet and squeezed for excess water, they were applied to the girdle and then encased firmly in a sheath of polyethylene plastic. A loose covering of aluminum foil completed the air-layer.

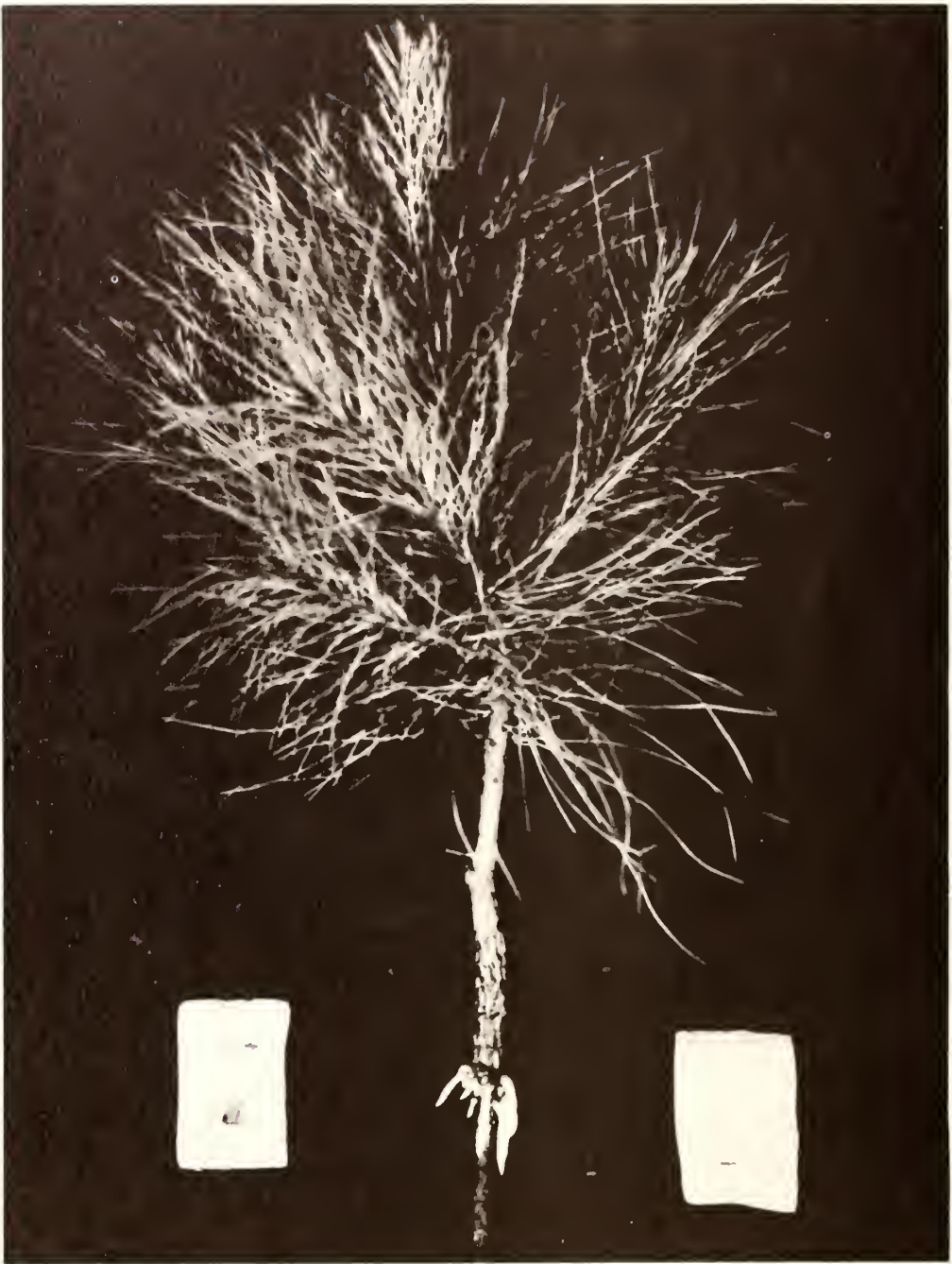


Figure 2.--Shortleaf pine seedling stem after 38 days air layering, with DuPont synthetic sponge as rooting medium.

Actually the roots did not enter the rather dense plastic material but grew in the cavities between the two blocks. A more "open" material molded into two fusiform halves would be ideal for this purpose and would simplify making the air-layer, particularly in the crowns of larger trees. Possibly sphagnum moss can be so processed by incorporating a binder and then molding. Or blocks of natural peat moss, sold as peat moss seedling pots, may be adapted for this use.

Shortleaf Versus Loblolly Pine

To test the difference between shortleaf and loblolly pine in rootability by air-layering, 10 seedlings of each were air-layered along their main stem by the "trough" method. Auxin treatment consisted of an application of Hormodin No. 3. The air-layers, made in June, were examined after 53 days and data on root development noted. As shown in table 1, 8 of 10 shortleaf and only 6 of 10 loblolly pine air-layers developed roots. This difference between the two species is even more effectively shown by the average number of roots per rooted seedling and by the average total length of main roots per rooted seedling. A representative seedling of each species is illustrated in figure 3.

Table 1.--Air-layered stems of 2½-year-old seedlings after 53 days;
shortleaf versus loblolly pine

Species	Stems air-layered	Stems rooted	Average roots per stem ^{1/}	Average total length of main roots per stem ^{1/ 2/}
	<u>Number</u>	<u>Number</u>	<u>Number</u>	<u>Inches</u>
Shortleaf	10	8	15.5	108.6
Loblolly	10	6	6.6	45.7

^{1/} Based on stems rooted.

^{2/} Does not include side roots--only those originating from callus zone.

With and Without Auxin

A group of shortleaf pine seedlings was air-layered upright along their stems to test the effect of auxin on rooting. Twelve seedlings were air-layered without the use of auxin, and 12 with an application of Hormodin No. 3. Begun early in August, the test was concluded after 45 days. The results (table 2) demonstrate that auxin greatly aided formation of roots. Other studies will determine the most favorable concentrations for pine of different ages.

Table 2.--Air-layered stems of 2½-year-old shortleaf pine seedlings after
45 days; effect of auxin on root development

Treatment	Stems air-layered	Stems rooted	Average roots per stem ^{1/}	Average total length of main roots per stem ^{1/ 2/}
	<u>Number</u>	<u>Number</u>	<u>Number</u>	<u>Inches</u>
Control	12	9	4.7	0.7
Hormodin No. 3	12	12	10.0	6.0

^{1/} Based on stems rooted.

^{2/} Does not include side roots--only those originating from callus zone.

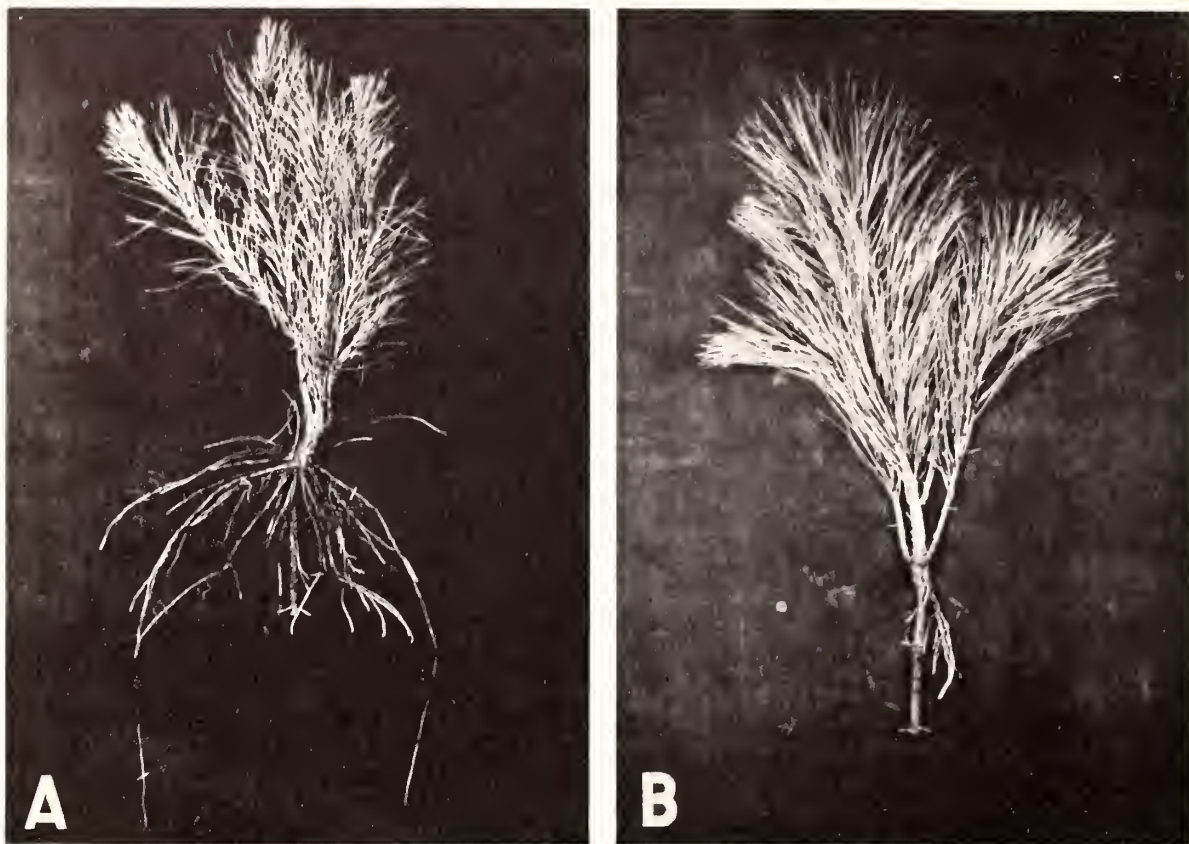


Figure 3.--Air-layered 2½-year-old shortleaf and loblolly pine. Former species exhibited much greater root forming ability. A, Shortleaf pine. B, Loblolly pine.

Air-Layering Grafted Pine

Branches on two shortleaf pine grafted trees, one made 8 and the other 9 years ago and now 10 to 12 feet in height, were air-layered using Hormodin No. 3. The original scion parents were 35 to 40 years old at the time of grafting. Of 11 air-layers made in June on the younger tree and 14 on the older tree, only one, on the latter, showed signs of root development after 65 days. The single successful air-layer had only one root and it was only 1/8 inch in length.

Thomas and Riker (5), rooting eastern white pine cuttings, also experienced poor rooting of cuttings taken from grafted trees made with scions from mature trees in the field. The grafted trees reacted like adult trees. Cech (3) has had good results with both cuttings and air-layers on young loblolly pine but little success with older trees. Others have encountered similar difficulties when attempting to propagate from older trees by these methods. The author feels that although current growth shoots on older trees are "young" in a developmental sense, their tissues, nevertheless, may also be considered physiologically "old" in that they contain elements of aging passed on and accumulated from growing season to growing season.

Air-Layering of Needle Fascicles

A modified form of air-layering needle fascicles or dwarf shoots was devised for possible use in vegetatively propagating shortleaf and other pines. For this work, 2½-year-old potted shortleaf pine seedlings again served as test plants. First, needle fascicles were thinned from a 3-inch length of stem 12 inches above ground to allow more working space for the remaining needles. Then, beginning about ¼ inch below the needle fascicle base, an upward cut was made with a sharp thin-bladed knife. This produced a slab of bark and wood from 1/2 to 5/8 inch long, 3/16 inch wide, and 1/16 to 1/32 inch thick, bearing a needle fascicle and attached solely at its upper point to the main stem. As many as 12 such slabs were made on individual stems along a 3-inch length. Finally, Hormodin No. 3 was dusted onto the cut surfaces and the treated area enveloped in moist sphagnum moss for 45 to 60 days.

Callus formation from each slab was excellent (fig. 4). And, from many of these callus areas one or two roots protruded (fig. 5), some being over 4 inches long, with side roots. Those slabs, both with and without roots, still bearing their needle fascicles were next severed from the stem and planted in a coarse sand-peat moss mixture in a warm greenhouse to force growth of the apical meristem. High humidity was maintained and, as this phase took place during fall and winter, day length was increased 3 hours by use of artificial light.

The method was first tried in August on 10 seedlings by means of the "trough" procedure previously discussed. After 42 days the stems were exposed and examined; the results for individual trees are presented in table 3. All needle fascicle slabs developed callus tissue but only 13 percent formed roots. Those of both groups still bearing needles were planted in the greenhouse. This included 10 slabs with roots and 66 slabs without roots. After 4 months, 9 in the first group were dead--one remained alive but showed no sign of top growth. In the second group, 44 of the 66 slabs died--of the remaining 22 only one formed roots after planting. All lacked any evidence of top growth.

A second trial was made in mid September on the upright stems of 9 shortleaf pine seedlings. This time ¼-inch lengths of toothpick were inserted under the slabs, which had been cut to separate them from the main stem and so prevent reunion. Hormodin No. 3 was dusted over the cut surfaces, sphagnum moss applied, and the whole enveloped in polyethylene plastic sheeting. No other covering was used.

Results after 65 days are seen in table 4. For all trees, 76 percent of the air-layered slabs formed callus tissue and 55 percent bore roots, many as long as 4 inches and with side roots. The piece of toothpick inserted under each slab helped in preventing union with the stem; several slabs with roots were quite free and were easily severed.



Figure 4.-- Needle fascicle air-layering on stem of shortleaf pine seedling. Shows callused tissue below fascicle bases before root initiation.

Table 3.--First needle fascicle air-layering on 2 $\frac{1}{2}$ -year-old shortleaf pine seedlings after 42 days

Tree number	Fascicle slabs	
	Air-layered	Forming roots ^{1/}
	Number	Number
1	9	1
2	12	0
3	10	0
4	10	0
5	10	3
6	10	0
7	10	5
8	10	0
9	10	4
10	10	0
Total	101	13
Percent		13

^{1/} All of the air-layers callused.



Figure 5.-- Needle fascicles with supporting tissue and roots, severed from seedling stem ready for planting.

Table 4.--Second needle fascicle air-layering on 2½-year-old shortleaf pine seedlings after 65 days

Tree number	Fascicle slabs			
	Air- layered	Callused		
		With roots	Without roots	Total
	Number	Number	Number	Number
1	8	5	0	5
2	8	4	2	6
3	6	3	2	5
4	7	5	0	5
5	5	3	0	3
6	8	6	0	6
7	6	3	2	5
8	6	3	1	4
9	8	2	6	8
Total	62	34	13	47
Percent		55	21	76

For this second test, 22 of the slabs with roots and 18 without roots and still retaining their needle fascicle were planted under conditions similar to those in the first study. Daylight was supplemented with 3 hours of artificial light. One month later, needle fascicles borne by 3 of the 22 slabs with roots showed beginnings of shoot growth from their bases. After 3 additional weeks, the new growth amounted to about 1½ inches for each (fig. 6). These complete little trees were transplanted into a soil-sand mixture and have continued growth since.

Admittedly, the method described has at present little practical value as a tool for the forest geneticist. It, in common with rooting and general air-layering of pine, has a serious limitation imposed by age, in that older trees are difficult if not impossible to propagate. In addition, there is the problem of forcing growth, or breaking dormancy, of the dwarf shoot or needle fascicle after roots have been induced by air-layering. These and other problems relating to this method must be solved before it can have wide use in forest genetics.



Figure 6.--Complete little tree produced by air-layering shortleaf pine needle fascicle. Air-layering continued 65 days, followed by 48 days in soil-sand mixture to break dormancy of the apical meristem in the base of the fascicle.

CONCLUSIONS

These experiments in air-layering shortleaf and loblolly pine show that the general branch method can be successfully applied to young trees. With older trees, this method shows much the same lack of success as the rooted cuttings method does; so reliance must still be placed on grafting for vegetatively propagating older material. The much better results obtained with air-layering than with the rooting of severed cuttings can, of course, be attributed to the continuity of xylem tissue from parent tree to the treated branch. The benefit may be interpreted as an adequate source of water and nutrients, permitting uninterrupted transpiration and normal metabolism. Full sunlight can be utilized for maximum photosynthesis to provide high accumulations of food in tissue from which roots originate.

Further studies, with the air-layering technique used as a tool, will attempt to find out why we get roots from the xylem-connected air-layered branch but few or none from the severed cutting. Eventually, it is hoped, techniques of rooting can be modified for successful use in forest genetics.

SUMMARY

Several modifications of the air-layering technique as applied to the stems of 2½-year-old shortleaf and loblolly pine seedlings and to branches of grafted shortleaf pine are described and discussed. The use of air-layering as a tool to study factors involved in the rooting of severed cuttings is considered.

A method of air-layering potted seedlings horizontally in a wooden trough filled with sphagnum moss is described.

Blocks of DuPont synthetic sponge were tried successfully as a substitute for sphagnum moss as the rooting medium in air-layering. A less dense material molded into fusiform halves is suggested.

Comparable 2½-year old potted shortleaf and loblolly seedlings were air layered to test species differences. Shortleaf exhibited a much greater root-forming ability than loblolly pine.

The effect of Hormodin No. 3 (0.8 percent indolebutyric acid) was tested in air-layering 2½-year-old shortleaf pine seedlings. Application of this auxin greatly aided root formation.

Two shortleaf pine grafted trees, with grafts made 8 and 9 years ago from scion material of adult trees, were air-layered with poor results. The almost complete lack of success is attributed to physiologic age of the tissue.

A method of air-layering pine needle fascicles is described and the results for 2½-year old shortleaf pine seedlings are presented. In one trial, 55 percent of tissue slabs bearing needle bundles produced roots. Three such "rooted" fascicles, or dwarf shoots, broke dormancy and commenced shoot growth to become complete, fully established little trees.



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